PRELIMINARY NOTE

BBA 41134

Effect of monovalent cations on oxidative phosphorylation in submitochondrial particles

Intact mitochondria are relatively impermeable to monovalent cations (see ref. 1) and their addition to freshly isolated mitochondria has no significant effect on the efficiency of oxidative phosphorylation. However, in the presence of agents like valinomycin[2], gramicidin[3] and the actins[4], which induce an increase in the permeability of mitochondria towards monovalent cations, and of an oxidizable substrate (or ATP), the ions are transported into the mitochondria against a concentration gradient and this is accompanied by uncoupling of respiration from phosphorylation[2–4]. It is generally agreed that the site of the permeability barrier and of the transporting system for monovalent cations is the inner mitochondrial membrane. In this communication, the effect of monovalent cations on oxidative phosphorylation in submitochondrial particles is reported. The particles were obtained by sonication of beef-heart mitochondria in the presence of Mg^{2+} and ATP (ref. 5). These particles are considered to consist of vesicles of the inner membrane turned "inside out"[6].

Table I shows the effect of a number of monovalent cations on respiration and oxidative phosphorylation in submitochondrial particles utilizing β-hydroxybutyrate as substrate. All the cations tested, including $Tris^+$, inhibited phosphate esterification.

TABLE I

EFFECT OF MONOVALENT CATIONS ON OXIDATIVE PHOSPHORYLATION IN Mg-ATP PARTICLES FROM BEEF-HEART MITOCHONDRIA UTILIZING β-HYDROXYBUTYRATE

The reaction mixture (final volume, 1 ml; final pH, 7.5) contained 200 mM sucrose, 3 mM $MgCl_2$, 1 mM EDTA, 10 mM P_i (potassium salt), 20 mM glucose, 5 units hexokinase, 8 mM potassium β-hydroxybutyrate, 1 mM NAD^+ (potassium salt), 0.1 mM ADP and Mg-ATP particles (5.0 mg protein). Incubation time, 14 min. Temp., 25°. Oxygen uptake was measured manometrically; phosphate esterification was measured by determining glucose 6-phosphate enzymically with glucose-6-phosphate dehydrogenase[7], correction being made for adenylate kinase activity. The submitochondrial particles were prepared as described in ref. 5, except that 1 M KOH was used instead of 1 M Tris to neutralize the beef-heart homogenate.

Addition	*Concentration (mM)*	*ΔO (μatoms)*	*ΔEsterified P (μmoles)*	*P:O*
None	—	2.20	1.72	0.78
KCl	50	2.20	1.13	0.51
KCl	100	2.29	0.77	0.34
NaCl	50	1.94	1.11	0.57
NaCl	100	2.34	0.85	0.36
NH_4Cl	50	2.28	1.23	0.54
NH_4Cl	100	2.45	0.87	0.35
LiCl	50	2.24	1.08	0.48
LiCl	100	2.22	0.72	0.32
Tris–HCl	50	1.89	0.91	0.48
Tris–HCl	100	2.23	0.50	0.22

The extent of the inhibition depended on the concentration of the cation (Table I and Fig. 1). Respiration, on the other hand, was not significantly affected under these conditions. The net result was a fall in the P:O ratio. Similar results were obtained with succinate as substrate.

Fig. 1 shows that valinomycin enhanced the effect of K^+ in lowering the P:O ratio. At the lowest concentration of K^+ used in this experiment (22.5 mM), the P:O ratio was 0.89 in the absence and 0.87 in the presence of valinomycin. At 205 mM K^+, the values were 0.23 and 0.08, respectively.

The nature of the inhibition by K^+ of the rate of phosphate esterification was examined kinetically. The Lineweaver–Burk plot of Fig. 2 shows that the inhibition was competitive with respect to ADP*. In this experiment, carried out in the presence of 66 mM $Tris^+$, the K_m for ADP was 40 μM in the absence of K^+ and 80 μM when 30 mM K^+ was added.

The finding that monovalent cations (including $Tris^+$) uncouple oxidative phosphorylation and that in the case of K^+ this effect is enhanced by valinomycin, which mediates the transport of K^+ (refs. 1, 2, 8), indicates that the particles retain the capacity for the transport of monovalent cations (and possibly of other cations; see ref. 9). SMITH AND BEYER[10] have reported that K^+ ($\pm$ valinomycin) has no effect on oxidative phosphorylation in electron heavy transport particles (ref. 11) from beef-heart mitochondria. However, their data show that the addition of 30 mM K^+ lowers the P:O ratio from 1.4 to 1.2 with NADH as substrate.

BYGRAVE AND LEHNINGER[12] have reported a value of 300 μM for the K_m for ADP in oxidative phosphorylation in submitochondrial particles. Our results suggest that the high value found by these authors may be due to the fact that they suspended

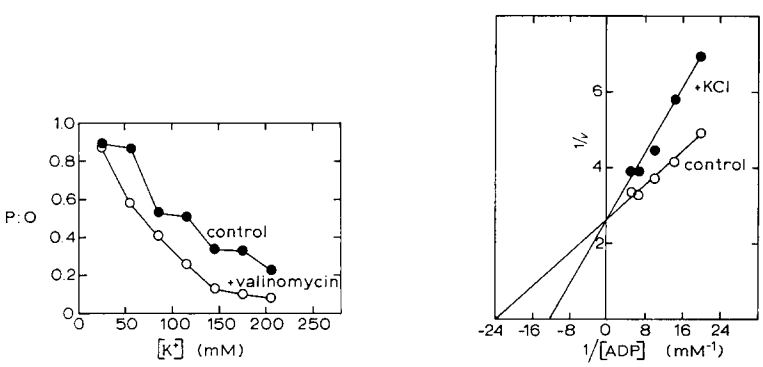

Fig. 1. Effect of different concentrations of KCl ($\pm$ valinomycin) on oxidative phosphorylation in Mg-ATP particles from beef-heart mitochondria utilizing β-hydroxybutyrate. Conditions as in Table I, except that 1.5 mg particle protein were used and the reaction time was 20 min. Where present, 0.1 μg valinomycin was used.

Fig. 2. Lineweaver–Burk plot of the rate of phosphate esterification *versus* the concentration of ADP in the presence and absence of KCl. The reaction mixture contained 200 mM sucrose, 50 mM Tris–HCl, 5 mM $MgCl_2$, 1 mM EDTA, 10 mM P_i (Tris salt), 20 mM glucose, 0.1 mM ADP, 1 mM NAD^+ (potassium salt), Mg-ATP particles (0.52 mg protein) and (where present) 30 mM KCl. Incubation time, 4 min. Other experimental conditions as described in Table I. v_{max} = 95 nmoles esterified P/min.

* It should be noted that v in Fig. 2 represents the rate of phosphate esterification, and not the P:O ratio.

the particles in a KCl *plus* NaCl medium. It is clear that the concentration of monovalent cations (including $Tris^+$) in the medium should be kept low in studying the efficiency of oxidative phosphorylation in submitochondrial particles and the affinity of the system for ADP. It should be noted that there is a requirement for a monovalent cation for the dinitrophenol-induced ATPase in intact rat-liver mitochondria[13–15] and that this may be satisfied not only by K^+ and Na^+ (ref. 13) but also by a range of other monovalent cations, including $Tris^+$ (see ref. 15).

The lack of an absolute valinomycin requirement for the inhibitory effect of K^+ on oxidative phosphorylation in these submitochondrial particles suggests that sonication renders the membrane so leaky that, even in the absence of valinomycin, an energy-dissipating transport of monovalent cations, perhaps cyclic in nature, will set in. It is conceivable that in submitochondrial particles the efficiency of oxidative phosphorylation (*cf.* ref. 6) as well as the affinity for ADP and P_i in the phosphorylating system largely depends upon the extent to which cyclic transport of monovalent cations and possibly other ionic species competes with phosphorylating reactions for energy conserved in the oxidoreductions of the respiratory chain.

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Received July 8th, 1968
Revised manuscript received October 2nd, 1968

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